

WHAT IS CLAIMED IS:

1. A method for separating oligonucleotides, said method comprising:

5 a) providing a plurality of oligonucleotides, said plurality of oligonucleotides comprising at least one bifunctional oligonucleotide and at least one non-bifunctional oligonucleotide, wherein each said at least one bifunctional oligonucleotide comprises a first separation tag attached to a first end of said at least one bifunctional oligonucleotide and a second separation tag attached to a second end of said at least one bifunctional oligonucleotide, and wherein cleavage of said first or said second separation tags yields an oligonucleotide having a 3'hydroxyl moiety;

10 b) contacting said plurality of oligonucleotides with a separation medium under conditions effective for adhering said at least one bifunctional oligonucleotide to said separation medium; and

c) selectively eluting at least one non-bifunctional oligonucleotide.

15 2. The method of claim 1, wherein said non-bifunctional oligonucleotides comprise depurinated or truncated oligonucleotides.

3. The method of claim 1, wherein either of said first or said second separation tags interacts with said separation medium via a noncovalent interaction.

20 4. The method of claim 1, wherein either of said first or said second separation tags interacts with said separation medium via a covalent bond.

5. The method of claim 4, wherein said covalent bond is selected from the group consisting of disulfide, hydrazo, alkoxyamino, and reactive carbonyl bonds.

25 6. The method of claim 3, wherein said noncovalent interaction is selected from the group consisting of hydrophobic, hydrophilic, hydrogen bonding, metal-complexing, ionic, and antigen-antibody interactions.

7. The method of claim 1, wherein said first and said second separation tags are different.

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8. The method of claim 1, wherein either of said first or said second separation tags comprise a separation unit selected from the group consisting of alkoxytrityl, alkoxypropyl, alkyldithioformacetal, methylthioalkyl, derivatives of mercaptodimethoxytrityl or mercaptotrityl, and a hydrocarbon chain introduced in a form of a linear or branched diol, and combinations thereof.
9. The method of claim 8, wherein said alkoxytrityl is selected from the group consisting of 4-decyloxymethoxy trityl (C₁₀Tr), 4-hexyloxymethoxytrityl (C₆Tr), dimethoxytrityl (DMTr), and monomethoxytrityl (MMTr).
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10. The method of claim 8, wherein said alkoxypropyl comprises 4-octadecyloxyphenylxanthyl (C₁₈-PX).
11. The method of claim 8, wherein said separation unit comprises a derivative of a mercaptodimethoxytrityl or mercaptotrityl.
12. The method of claim 8, wherein said separation unit is a methylthioalkyl moiety.
13. The method of claim 8, wherein said separation unit is a hydrocarbon chain introduced in a form of a linear or branched diol.
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14. The method of claim 1, wherein a cleavable unit of either of said first or said second separation tags is selected from the group consisting of acid labile, fluoride ion labile, photolabile, redox labile, and electrophile labile moieties.
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15. The method of claim 14, wherein said redox labile moiety comprises a dithioformacetal moiety.
16. The method of claim 1, wherein a cleavable unit of either of said first or said second separation tags comprises a siloxyl or disiloxyl moiety.
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17. The method of claim 1, wherein said separation medium is selected from the group consisting of affinity, hydrophobic interaction, hydrophilic interaction, metal-chelating, ion exchange, covalent coupling, and antigen-antibody affinity separation media.

18. The method of claim 1, wherein said separation medium is an ion exchange separation medium.

19. The method of claim 1, wherein said separation medium is a reversed phase separation medium.

5 20. The method of claim 1, wherein said separation medium is a mixed-mode type separation medium.

21. The method of claim 20, wherein said mixed-mode type separation medium comprises reversed phase and ion exchange separation media.

22. The method of claim 20, wherein said mixed-mode type separation medium comprises a
10 covalent coupling separation medium.

23. The method of claim 22, wherein said covalent coupling separation medium is based on the formation of a disulfide bond.

24. The method of claim 1, wherein said method further comprises eluting said at least one bifunctional oligonucleotide.

15 25. The method of claim 1, wherein said separation medium comprises a first separation medium and a second separation medium, said first separation medium effective for adhering to said first separation tag and said second separation medium effective for adhering to said second separation tag.

26. The method of claim 1, said method further comprising:

- 20 d) cleaving either said first separation tag or said second separation tag; and
 e) eluting an oligonucleotide lacking said non-cleaved separation tag.

27. A method for separating oligonucleotides, said method comprising:

- a) providing a plurality of oligonucleotides, wherein said plurality of oligonucleotides
comprises at least one bifunctional oligonucleotide and at least one non-bifunctional
25 oligonucleotide, and wherein each said at least one bifunctional oligonucleotide comprises a

2025 RELEASE UNDER E.O. 14176

first separation tag attached to a first end of said at least one bifunctional oligonucleotide and a second separation tag attached to a second end of said at least one bifunctional oligonucleotide, and wherein cleavage of said first or said second separation tags yields an oligonucleotide having a 3' hydroxyl moiety;

- 5 b) contacting said plurality of oligonucleotides with a separation medium under conditions effective for adhering said at least one bifunctional oligonucleotide to said separation medium;
- c) eluting non-bifunctional oligonucleotides lacking said first separation tag without eluting said bifunctional oligonucleotides;
- 10 d) cleaving said first separation tag from the oligonucleotides retained on the separation medium; and
- e) eluting non-bifunctional oligonucleotides lacking said second separation function.

28. The method of claim 27, wherein said cleaving step is facilitated using TBAF.

29. The method of claim 27, wherein said cleaving step is facilitated using an acid.

15 30. A composition comprising:

 a) a plurality of oligonucleotides, each said oligonucleotide comprising a first separation tag attached to a first end of said oligonucleotide and a second separation tag attached to a second end of said oligonucleotide, wherein cleavage of said first or said separation tags yields an oligonucleotide having a 3' hydroxyl moiety; and

20 b) a separation medium, said plurality of oligonucleotides adhering to said separation medium.

31. The composition of claim 30, wherein said separation medium comprises a first separation medium and a second separation medium, said first separation medium and said second separation medium being different separation media.

25 32. The method of claim 1, wherein a cleavable unit of either of said first or said second separation tags comprises an alkylthiomethyl moiety.

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33. The method of claim 1, wherein a cleavable unit of either of said first or said second separation tags comprises a hydrocarbyldithiomethyl moiety.

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